REMARKS

A minor error in claim 244 which was presented in Applicants' September 18, 1995 Preliminary Amendment has been corrected above. Inadvertently, the term "nucleotide" was substituted for "oligo- or polynucleotide." With the proper recitation of "oligo- or polynucleotide" having been restored, claim 244 now properly depends from claim 243, the latter also reciting the "oligo- or polynucleotide." Except for this minor amendment, the claims presented for examination in this application continue to be represented by claims 204-224 and 227-258.

The Rejection Under 35 U.S.C. §103

In their September 18, 1995 Second Preliminary Amendment, Applicants addressed the obviousness rejection set forth in the October 4, 1994 Office Action issued in the parent application (Serial No. 08/046,004, filed on April 9, 1993). In addition to the remarks which appear on pages 14-16 of their September 18, 1995 Second Preliminary Amendment, Applicants would like to present the remarks below.

In the cited Kourilsky document, GB 2 019 408[1] (published on October 31, 1979), the following is disclosed beginning on page 1, line 61, and continuing through page 2, line 6:

> The method of detection according to the invention of the possible presence or of the characterization of a sequence or particular fragment of nucleic acid, notably of a gene, even of the whole nucleic acid in a complex sample of nucleic acids, by contacting the sample, if necessary after prior denaturation of the nucleic acid under study, with a probe comprising a complementary nucleic acid, capable of being hybridized with the nucleic acid sequence or the nucleic acid sought, is characterized in that the reagent or probe used is a probe modified chemically by coupling or for its coupling with an enzyme prior or subsequent to the hybridization reaction, the possible presence of nucleic acid sequence or of the nucleic acid sought being revealable by the action of the thus-transformed hybridization product of the probe and of the sequence or of the nucleic acid sought, on an enzyme substrate.

[1] **Applicants note that a corresponding application to Kourilsky's priority document (FR 7810975) was filed in the U.S. Patent and Trademark Office on April 13, 1979 and it eventually issued on April 8, 1986 as Kourilsky et al., U.S. Patent No. 4,581,333. A copy of the aforementioned '333 patent was previously submitted as Exhibit 52 in Applicants' August 22, 1994 Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.97-1.98 filed in the parent application.**

Later in lines 11-15 on page 2, Kourilsky et al. disclose:

> In a preferred embodiment of the application of the invention, the probe is modified by a chemical group capable of forming a stable complex with the enzyme or a molecule itself bound stably to the enzyme. Advantageously, the above-said chemical group and the above-said molecule are respectively constituted by biotin and avidin or vice versa, the enzyme being itself advantageously constituted by ß-galactosidase.

In apparent reference to the above-described preferred embodiment, the inventors state on page 2, lines 37-40:

> When the chemical modification of the probe is carried out by means of biotin, it is possible to resort to the technique described by Manning et coll. in the already mentioned publications ["A New Method of *in situ* Hybridization," *Chromosoma (Berl.) 53*, 107-117 (1975), Springer-Verlag 1975 and "A Method for Gene Enrichment Based on the Avidin-Biotin Interaction. Application to the *Drosophila* Ribosomal RNA Genes", *Biochemistry*, Vol. 16, No. 7, 1364-1369, 1977], through cytochrome C, notably in the proportion of one molecule of biotin on the average for about 100 nucleotides.[2]

In lines 41-43, the inventors continue:

> Advantageously, recourse is then had for labelling the hybrid by the enzyme, to the product resulting from the coupling of avidin and the enzyme, notably ß-galactosidase, by the Avrameas method ("Immunochemistry", 1969, *6*, 43-52).[3]

Finally, in some "experimental" discussion on page 3 (lines 28-29), Kourilsky et al. state:

> 1 µg of ribosomic RNA labeled with biotin by means of cytochrome, prepared by the technique of Manning & Coll., is added to the denatured DNA solution. . .

In reading Kourilsky's disclosure, it is patently clear that at most two techniques are envisioned for coupling ß-galactosidase to nucleic acid, the Manning technique involving cytochrome C as set forth in the two Manning publications, and perhaps the Avrameas technique which relies on glutaraldehyde as a coupling agent for conjugating enzymes to proteins as described in the 1969 Immunochemistry paper.

[2] The corresponding Kourilsky '333 U.S. patent also refers to these two Manning publications in column 2, lines 17-23.

[3] The Avrameas publication also cited in Kourilsky's '333 U.S. patent in column 3, lines 56-57.

For the Examiner's convenience and review, copies of Manning's 1975 and 1977 publications, and Avrameas' 1969 publication are attached to this Supplemental Amendment as Exhibits 1, 2 and 3, respectively.

The 1975 and 1977 Manning publications (Exhibits 1 and 2) are directed to gene mapping and gene enrichment techniques, respectively. As noted in the 1977 Manning publication (Exhibit 2), the radioactive labeled "*E. coli* DNA, coupled with cytochrome *c*-biotin, was prepared as described previously" (citing Manning et al. 1975a [that is, the 1975 Chromosoma paper (Exhibit 1) with some modifications. See 1977 Manning publication (Exhibit 2), page 1365, right column, first full paragraph.

In the 1975 Manning publication (Exhibit 1), the technique for coupling RNA to biotin-labeled cytochrome c involves terminal labeling of the sugar. The hydroxyl groups on the terminal sugar at the 3' end of RNA are oxidized and converted to the dialdehyde which is reactive with an amine. Cytochrome c is useful for Manning's purposes because it is spherical in shape, having fourteen (14) amine groups, one of which can react with the thus-formed dialdehyde and some others of which biotin can be incorporated as a label. Thus, Manning's disclosure is directed to the labeling the terminal sugar of RNA, and it is even questionable whether the terminal ribonucleotide is labeled as a nucleotide because the ribose sugar has been grossly changed by its convertion to a morpholine. What is unquestionable in Manning's disclosures is the lack of phosphate involvement in their labelling technique.

Avrameas (Exhibit 3) disclose the conjugation of enzymes with proteins using glutaraldehyde as the coupling agent. The free amino groups of proteins are said to participate in the cross-linking reaction with glutaraldehyde. See Avrameas (Exhibit 3), page 46, last three lines, through page 47, first two lines. Thus, the Avrameas publication itself is not even concerned with nucleic acid.

In view of the foregoing remarks, submitted exhibits and their previous remarks presented in their September 18, 1995 Second Preliminary Amendment, Applicants respectfully urge that the previous obviousness rejection under 35 U.S.C. §103 be thoroughly reconsidered before it is applied again to the instant claims.

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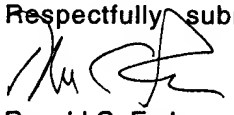
No fee is believed due for this Supplemental Amendment, the fees of $730.00, $220.00 and $506.00 [total of $1,456.00] having been paid with previous filings. In the event that any fee is due, however, The Patent and Trademark Office is hereby authorized to charge the amount any such fee to Deposit Account No. 05-1135, or to credit any overpayment thereto.

Early and favorable action on the claims presented for examination is courteously solicited.

Respectfully submitted,



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